VOLUME 4 JULY, 1934 NUMBER 4

American Journal of Clinical Pathology

OFFICIAL PUBLICATION
THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

CONTENTS

PUBLISHED BI-MONTHLY BY THE WILLIAMS & WILKINS COMPANY
MOUNT ROYAL AND GUILFORD AVES., BALTIMORE, U. S. A.

Made in United States of America

Entered as second-class matter, January 7, 1931, at the Post Office at Baltimore, Md., under the act of March 3, 1879

THE RÔLE OF THE PATHOLOGIST IN THE CANCER PROBLEM*

ALVIN G. FOORD

Pasadena Hospital, Pasadena, California

In 1931, Dr. C. C. Little in one of the International Contributions to the volume of the Annals of Surgery dedicated to James Ewing, made the statement that there are four great fields of work in which new advances are necessary in the control of cancer:

(1) More satisfactory and complete methods of classifying and of differentiating between the various types and degrees of cancerous growth will have to be established.

(2) Best possible methods of treatment must constantly be subjected to close scrutiny with the view in mind of improvement and refinement and of the development of new lines of attack.

(3) The immensely important work of attempting to decrease the incidence of cancer by education of laymen must be carried on.

(4) Research as to the cause or causes of uncontrolled cancer growths must be fostered.

He further wrote:

In all these problems the need of larger numbers of better trained medical men is paramount. Because of many reasons, among which the difficult and discouraging nature of cancer itself is undoubtedly one, the medical schools and active medical profession give much too little attention to training would-be or young doctors in the problem of cancer. Instead of recognizing the challenge offered by cancer as being an outstanding menace about which pathetically little is known and for the combating of which the utmost coöperation is needed, the medical profession fights shy of grappling with the problem and looks for easier foes to conquer. This attitude will have to be corrected. The spread of popular interest in cancer, coupled with a marked decrease in ignorant fear and superstition concerning it, will undoubtedly in the near future result in a

---

*Presidential Address read before the Thirteenth Annual Convention of the American Society of Clinical Pathologists, Cleveland, Ohio, June 8 to 11, 1934.

popular demand by the laity for better equipped medical men to cope with the situation. Then, if not before, medical schools and the medical profession as a whole will take the long overdue steps toward more up to date and extensive training of their personnel.

In the last few years an active response has been made to this challenge, and the fight against this disease has been waged with a vigor not known in the past, and throughout most States a sense of cancer-consciousness is being instilled into the medical profession. Most pathologists have fallen into line in this great battle and are endeavoring to aid in the first three of the methods of attack laid down by Dr. Little, and a few are able to carry on research on the cause of cancer. Some pathologists, however, have failed to realize their key position and have been made the object of attack by various critics.

I am not in accord nor do I believe this audience agrees with a statement in a recent editorial in the January, 1934, issue of the American Journal of Cancer, which ends with this paragraph:

> What medicine needs today is, first, personality, and second, wide training, both in the laboratory and the clinical aspects of the art. The lack is more often in the laboratory phase.

Personality certainly plays its part, but I feel certain that the status of pathological diagnosis of cancer is at present far ahead of the clinical aspect of diagnosis, and far superior to the average treatment afforded the cancer patient. That poor pathological diagnoses are made in some places in this country, none can deny, but by using the well-trained pathologists now available in the manner suggested by this Society and the American College of Surgeons, namely, by having a pathologist serve more than one hospital in smaller cities, conditions would be improved immensely and the untrained men would have to brush up or quit. This method would not correct the entire situation at once, but if profitable financial futures are guaranteed young men by hospitals and support by their confreres in clinical medicine, an increased supply of better trained men will be furnished to those places where they are needed. I believe that organizations endeavoring to strengthen scientific medicine and particularly labo-

ratory diagnosis should exert their major efforts on delinquent hospitals and lukewarm clinicians so they might arrange for satisfactory financial support of good pathologists. This should be done rather than endeavoring to change the entire scheme of the practice of pathology by advocating that it be practiced by men whose plan is to use the position as pathologist as a mere stepping stone toward clinical medicine or surgery.

Those already in the field have abundant opportunities to aid in the cancer campaign and can serve as others cannot on our hospital staffs. Our position is the corner stone of the problem in the individual patient and not merely that of diagnosing the type of tumor under a microscope, perhaps several days after a surgical specimen is sent to a laboratory. It should begin in many cases before clinical diagnosis is made or treatment started. We should be, as Dr. Simpson stated last year in his presidential address, consultants, paid or otherwise, and if our clinical training has been slighted in the past, tumor cases will certainly furnish material for increasing our own diagnostic abilities. Following this we should be on hand in the operating room to give opinions on gross tissue specimens followed by frozen sections if necessary, in order that proper treatment may be established immediately. It has been my experience that the better surgeons are anxious for all the detailed information they can get. Also, more patients every year are asking for prompt diagnosis on their tumors before mutilating surgery is done. We must furnish this service to accomplish properly our work.

I should not need to mention to a group of pathologists that in case of death a necropsy should be performed on all cancer patients, but a look at the post mortem statistics in many hospitals will convince any one that many are missed. It is common experience that post mortem consent is easier to obtain in cases dying from cancer than from other causes of death, and often a little gentle prodding from the pathologist will induce an attending physician or his assistant to gain the necessary permission. Particularly important are post mortem studies of cancer patients treated by roentgen ray or radium, especially in those given the stepped up dosages used in well equipped clinics at the present

time. Without such studies we can never know what is being done by these agents. Incidently it is important that the pathologist acquaint himself with the picture presented by heavily radiated tissues otherwise he may make pitiful mistakes in cases of biopsies or surgical specimens from treated cases.

Furthermore, the pathologist should be one of the most active individuals in the cancer clinic in his hospital, or, if none exists, he should endeavor to impress on his staff the necessity for such a clinic. He, having no axe to grind and being in a neutral position, can often serve as the arbitrator in disputes between the radical surgeon and the over-enthusiastic radiotherapeutist, or between the over-pessimistic internist and the zealous dermatologist. With a little extra work on his part he can demonstrate gross material and microscopic preparations of tissues removed from patients before or after presentation before the clinic. Most clinicians are anxious to see such material if presented properly.

In smaller hospitals particularly, the pathologist can aid a great deal in teaching his own staff by summarizing and analyzing the records and end results in cases of malignancy treated in his own hospital, where such attempts by one or more clinicians might not be favored by some of the staff because of professional rivalry, personal or other reasons. By such analysis the results of various methods of treatment can be evaluated, mistakes in diagnosis and therapy can be impersonally discussed, and pertinent data can be obtained as to the variation in the clinical course depending on the type and grade of the tumors, the location and extent of the involvement, et cetera. This material may not be as abundant as in some larger institutions, but oftentimes it is more interesting, since the cases are those actually seen by the local men, including not only the man who treats only an occasional case but also those who handle larger numbers but never stop long enough to take stock of their final results.

Also the pathologist, realizing the necessity for more information about cancer among clinicians and pathologists alike, should see to it that case reports or papers on malignant disease be presented more often on the programs of staff meetings or those of local, county, state, or even national organizations. He

should take the time and effort to work up interesting cases, including gross and microscopic photography for presentation by the clinician or himself, and although it may cost him a large amount of labor and effort, in the long run the knowledge gained will be worth the price, and the cementing of friendship with his clinical confreres will more than repay him.

Before closing, may I mention a few methods which we as a Society should support or sponsor in disseminating information on the pathological diagnosis of tumors to pathologists. Needless to say, the best way is to take time off and study at some of the larger clinics here or abroad, but in these days this cannot be done except by a few. Failing that, we can bring the tissues to the pathologist. This we have started in the form of our Tumor Registry under Dr. Brines' committee who are collecting slides accompanied by histories, et cetera of tumors from all organs of the body. These will be sent out as loan slides (five or more sets will be available) and should serve as topics of discussion for small groups of men. This registry is in no way to conflict with the Bone Tumor Registry of the American College of Surgeons or the Lymphatic Tumor Registry of the American Association of Pathologists and Bacteriologists. On the contrary, support of these registries is more than desired since material sent to them serves as a source of information in the reports of the study of the large numbers submitted, and also the individual pathologist who sends in a case has the benefit of the best consultation in the country.

Furthermore, our state counsellors or men chosen by them should organize small units of pathologists in places where no regular pathology society exists, as has been done in some of the states already. By a little effort arrangements can be made for visits by men of larger experience, and in turn the visitor, I am sure, will be repaid by seeing material fully as interesting as in his own hospital or university.

Another scheme which works splendidly and which might serve as a model for other states is the holding of semiannual round table meetings as is done under the auspices of the Cancer Commission of the State Medical Society in California. Eight or

ten cases are presented clinically, gross specimens or lantern slides are demonstrated, and then the slides of the tissue are examined, each man having his own slide, followed by a vote on the diagnosis. The man presenting the case then discusses his own diagnosis, followed by general discussion. An average of thirty-five to forty pathologists attend the meetings and return with a full set of slides of rather difficult tumors for future reference. These meetings, if they serve no other purpose, serve as a stimulus to those men who have poorly made slides to pay attention to this very vital point in tissue diagnosis.

Finally, in behalf of the tumor patients of this country who come to medical men, may I recommend that we all consider the subject of tumors more fully than ever, not only from the standpoint of how many mitotic figures there are in a microscopic field but from the viewpoint of curing the particular patient we are interested in, and from the general viewpoint of the handling of the entire cancer problem. We have as yet only scratched the surface. Let us dig deeper, help when we can, and above all, furnish accurate information and proper diagnoses from our own laboratories, scrutinize carefully new developments of treatment, stress cancer to our students and keep our colleagues alive to the subject.

CLINICO-PATHOLOGICAL RELATIONSHIP IN COMMON BREAST LESIONS*

FREDERICK H. LAMB

1001 American Bank Building, Davenport, Iowa

The statistical portion of this survey is based on the clinical and laboratory records of 255 patients admitted to St. Luke's Hospital, Davenport, Iowa, and St. Anthony's Hospital, Rock Island, Illinois, for the diagnosis and treatment of breast disease, within the ten year period from 1924 and 1933, inclusive. During this decade the total combined admissions were 34,464, exclusive of dispensary and out patients, but including an undetermined number of readmissions.

Since the average number of patients in each institution has been approximately the same, the first five of the following tables are arranged so that the statistical data in both series may be compared. In the remaining two tables, the totals are combined.

From table 1, it will be seen that nine patients were considered to have inoperable malignancies, and that thirteen, presumably with carcinomas, were treated nonsurgically, although there was no histological verification, and both groups have been eliminated from further consideration in this report. In 214 cases from which histological examinations were made, no pathological lesion was recognized in nine instances, other than simple or involutional atrophy.

As indicated in table 2, the incidence of malignant disease comprised about one-third of the total number; benign tumors, a little less than one-half; cystic disease, approximately 11 per cent, and inflammatory lesions 6 per cent.

In this series, the malignancies were not classified originally with a view to grading the degree of anaplasia, except in so far

* Presented at the Clinical-Pathological Conference, February 8, 1934, St. Anthony's Hospital, Rock Island, Illinois.

as the general histological architecture of the neoplasm might serve that purpose. The terms adenocarcinoma, scirrhous and medullary carcinoma have long been used to designate respectively the tendency to gland formation, a relative abundance of

TABLE 1

	ST. LUKES	ST. ANTHONY	TOTAL
Breast cases (1924–1933)	137	118	255
Incised and drained	2	5	7
Inoperable (carcinoma)	4	5	9
Treated nonsurgically	1	12	13
Examined histologically	121	93	214
Acquired anomalies (adult hypertrophy)	3	0	3
No lesions recognized*	5	4	9

* Except simple or involutional atrophy.

TABLE 2

INCIDENCE OF VARIOUS LESIONS

LESIONS	ST. LUKES	ST. ANTHONY	TOTAL	
			Number	Per cent
Malignancies	42	31	73	35.6
Benign tumors	48	42	90	43.9
Cystic disease	14	9	23	11.2
Inflammatory lesions:				
Acute pyogenic (multiple abscesses Chronic mastitis (exudative and fibrous	5	7	12	5.8
Tuberculosis	1	0	1	0.5
Anomalies:				
Gynecomastia	2	0	2	1.0
Adult hypertrophy	3	0	3	1.5
Unclassified	1	0	1	0.5
Totals with recognized lesions	116	89	205	

fibrous connective tissue stroma, and solid masses of cancer cells with but little fibrous stroma. Deductions drawn from early studies in histological prognosis implied that adenocarcinomas were the least malignant, scirrhous types occupied an intermediate position, and medullary carcinomas were the most malig-

nant.[2] Follow-up statistics have not always borne out these implications, particularly with reference to differences between the scirrhous and medullary types. In reviewing my descriptions of histologic sections in individual cases, I find that I have referred frequently to the number of mitotic nuclear figures, to the chromatic character of the nuclei, pleomorphism, and variation in size of both cells and nuclei, to the diffuse or circumscribable character of carcinomatous infiltration, to the presence of inflammatory or lymphocytic infiltration, and to evidence of secretory activity of both parenchyma and stroma. These observations

TABLE 3

MALIGNANCIES

TYPE OF TUMOR	ST. LUKES	ST. ANTHONY	TOTAL
Scirrhous	17	10	27
With extensive fibrosis	2	0	2
Far advanced	2	1	3
Adenocarcinoma	3	4	7
Papillary cyst	0	3	3
Partly scirrhous, alveolar, or medullary	8	5	13
Medullary	7	5	12
Paget's disease of nipple	1	3	4
Sarcoma:			
Alveolar	1	0	1
Fibrosarcoma	1	0	1

were made in a purely objective manner, however, and without regard to any formula or formal system for histological prognosis. A correlation of these and other factors has been undertaken by the reëxamination of sections from the paraffin blocks in this series combined with a larger group of breast cases. There is at present, a wide difference of opinion among pathologists regarding the merits of histological grading.

Ninety cases (44 per cent) of the 205 cases in which pathological changes were recognized were classified as benign tumors. Solid adenofibromas made up about 60 per cent of the group of benign tumors, while cystadenofibromas and adenofibromas with widely dilated ducts accounted for approximately 25 per cent.

As a practical point, cystadenomas which contain blood, those lined by soft partly necrotic tissue, and those which have thick rigid walls should be carefully examined for evidences of malignant transition. Early carcinomatous change was observed in one cystadenoma in this series.

TABLE 4

BENIGN TUMORS

TYPE OF TUMOR	ST. LUKES	ST. ANTHONY	TOTAL
Adenofibroma:			
Peri- or intracanalicular	21	28	49
With myxomatous degeneration	2	0	2
With diffuse mastitis	3	1	4
Cystadenofibroma	12	8	20
With early malignant change	1	0	1
With chronic mastitis	1	0	1
Ectatic ducts only	2	0	2
Adenoma	3	2	5
Lipoma	3	3	6

TABLE 5

CYSTIC DISEASE AND INFLAMMATORY CHANGES

LESION	ST. LUKES	ST. ANTHONY	TOTAL
Cystic disease:			
Large and small cysts Adenomatous hypertrophy of lobules	14	9	23
Galactocele	1	0	1
Inflammatory changes:			
Acute, pyogenic (multiple abscesses)	1	5	6
Chronic mastitis (exudative and fibrous)	3	2	5
Tuberculosis	1	0	1

Twenty-four cases (12 per cent) of the total were regarded as cystic disease of the breast (Reclus's disease). In certain stages of development the distinctions between retention cysts, cystic disease, or cystic mastitis, and cystadenomas are not always clear cut. Usually the character of the stroma, and the absence of small satellite cysts help to differentiate cystadenomas from cystic mastitis. Retention cysts often contain a liquid or semisolid

milky fluid. That type of cystic disease in which there are solid masses of cells presenting microscopically an adenomatous hypertrophy of the lobules is of special interest since it may be mistaken for adenocarcinoma. This has been considered by some pathologists to be a precancerous lesion, yet it differs sharply from carcinoma in that the glandular hyperplasia retains a lobular arrangement. So far as can be determined from follow-up records in this small series there has been no instance of carcinoma developing after simple excision or mastectomy for cystic disease.

In table 6 an attempt has been made to correlate the incidence of palpable lymph nodes as reported in the clinical histories with the post-operative examination of tissues, and the mobility or

TABLE 6

ADENOPATHY AND MOBILITY

	GLANDS—PRE-OPERATIVE EXAMINATION			GLANDS— POST-OPERATIVE EXAMINATION			MOBILITY OF TUMOR		
	Present	Absent	Unreported	Present	Absent	Unreported	Fixed	Free	Unreported
72 malignancies	38	25	19	47	21	4	33	29	10
80 benign tumors	10	48	12	4	62	14	3	64	13
20 cystic disease	2	15	3	1	14	5	2	17	1

fixation of the various breast lesions as recorded in the clinical findings. A number of clinical records in the series had to be discarded due to incomplete data. Not all of the records from which table 6 is made up were as complete in this respect as might be desired; the deficiencies are indicated in the table in columns headed "unreported." For example, in the seventy-two malignancies, palpable lymph nodes were noted in the clinical histories in thirty-eight cases; absence of palpable nodes was noted in twenty-five cases; and no notation was made in nine cases. In the gross examination of the tissues, enlarged lymph nodes were found in forty-seven cases, no nodes were found in twenty-one, and in four instances notation was not made. The figures set down opposite the eighty benign tumors, and twenty cases of cystic disease are to be interpreted in the same

manner. Summarizing, in a general way, that portion of the table referring to adenopathy, it will be noted that palpable lymph nodes were found preoperatively at the time of examination in about one-half of the patients suffering from malignancies. Post-operatively enlarged nodes were found in approximately two-thirds of these cases. Enlarged lymph nodes were found rarely in association with the benign tumors and cases of cystic disease, although other palpable masses were occasionally mistaken for enlarged nodes.

Mobility, as the term is used in this table, refers not alone to nodular fixation or freedom of movement, but in order to simplify the analysis, it includes dimpling of the skin, retraction of the nipple and subdermal thickening. Only those breast nodules were classified as freely movable which were not fixed, and which were unaccompanied by retraction, dimpling and subdermal thickening. In twenty-nine cases (40 per cent) of the malignancies of this series, the neoplasm was freely movable at the time of examination. It should be remembered that malignant nodules centrally located within the breast remain movable until extension and infiltration involve the dermal or subdermal lymphatic spaces, or the underlying muscle fascia. It is an important point that these well known clinical signs are of diagnostic value only when they are present; their absence should not be taken as evidence against malignancy, especially in the case of an intramammary nodule.

There is possibly no better illustration of the advantage of coöperation between surgeon and pathologist, than in the management of breast disease. That coöperation is especially desirable in the most common manifestation of breast disease, namely the solitary mobile nodule. By means of the frozen sections, the character of its histologic structure may be determined within a few minutes, and the scope of the operation planned accordingly. Experience with frozen sections over a period of years has repeatedly demonstrated their practical worth. Moreover, exploratory operations should not be performed unless the surgeon is prepared either alone or with the aid of a pathologist to establish the diagnosis and proceed with the radical operation,

if carcinoma is found. The less experienced surgeon, who relies on his knowledge of gross pathology alone assumes an unenviable degree of responsibility.

From the standpoint of age incidence, about 85 per cent of the benign tumors occurred in patients under fifty years, while approximately the same percentage of malignancies were found in persons over forty years of age. The age incidence of cystic disease approximated that of benign tumors.

TABLE 7

AGE GROUPS IN THE COMBINED SERIES

AGES	MALIGNANCIES	BENIGN TUMORS	CYSTIC DISEASE
10–19	0	1	0
20–29	1	15	4
30–39	6	36	5
40–49	23	32	8
50–59	18	3	3
60–69	14	4	1
70–79	9	1	0
80–89	2	1	0

DIFFERENTIAL DIAGNOSIS

It is important to determine promptly and decisively the nature of breast nodules. There are but few, if any, valid reasons for such advice as "to wait and see what happens." It would be far safer for the patient if the physician were to assume that all breast nodules were malignant until proved otherwise.

Errors in diagnosis, and consequently the treatment based on that diagnosis if uncorrected, occur most commonly under three headings:

(1) A small, circumscribed, freely movable, isolated nodule without any sign of attachment to the skin or nipple occurring in a young woman often leads to the diagnosis of adenofibroma, when actually the nodule is malignant. As pointed out in the discussion under table 6, nearly 40 per cent of the malignant nodules in this series were movable and without skin attachment; and as shown in table 7, 12 per cent of the breast lesions in women under forty years of age were malignant.

(2) A slowly growing, firm, circumscribed mass unattached to the skin or underlying muscle, and without palpable axillary glands is assumed at times to be benign, on the basis of a long history. Actually such a nodule may well be a duct carcinoma, or slowly growing cystadenocarcinoma free of attachment in spite of its size.

(3) The presence in one or both breasts of more than one tumor throws the weight of evidence against malignancy, and favors the diagnosis of benign lesions.[1] Multiple tumors may be fibroadenomas, or still more likely some form of cystic disease. Pain and tenderness are less frequently associated with fibroadenomas than with cystic disease. A conservative operation may be planned therefore in the treatment of multiple painless nodules, and if at operation these tumors are found to be solid, local excision with a margin of normal tissue is adequate. On the contrary, mastectomy is the operation of choice in multiple cystic tumors. Both large and small cystic nodules are frequently surrounded by an area of smaller microscopic cysts extending for some distance into the mammary tissue. While the larger cysts are not likely to undergo malignant transition due to degeneration of the glandular epithelium, the epithelial cells of the smaller cysts are more active and probably more responsive to whatever stimulus induces malignant change (Schimmelbush's disease). Although malignant transition is uncommon, to remove the larger cysts and leave the smaller ones is to remove the more innocent lesion and leave behind the more dangerous one.[1]

SUMMARY

A knowledge of the clinico-pathological relationships in breast disease is the basis for its intelligent management. The simple classification of benign and malignant tumors, cystic and inflammatory disease covers the vast majority of lesions with which one may be confronted. Some of the natural limitations in the differential diagnosis of the most common breast lesion, namely the solitary mobile nodule, have been emphasized in a statistical way. It has been pointed out that multiple lesions in one or both breasts are likely to be benign solid tumors or a type of cystic

disease, and the rationale of simple excision in the former case and mastectomy in the latter has been explained. Personal experience in the immediate examination of breast tumors has demonstrated repeatedly the advantage of this form of coöperation between the surgeon and pathologist. An exploratory operation on the breast should not be undertaken unless one is fully prepared to perform a radical operation if malignant disease is found.

REFERENCES

(1) CUTLER, MAX: Benign lesions of the female breast simulating cancer. Jour. Am. Med. Assn., **101:** 1217–1222. 1933.

(2) EVANS, W. A.: Histological factors in the prognosis of mammary cancer treated by radical operation and X-rays. Am. Jour. Cancer, **19:** 328–338. 1933.

THE CAUSE OF LOCAL REACTIONS FOLLOWING THE ADMINISTRATION OF STAPHYLOCOCCUS BACTERIOPHAGE*

WALTER E. KING, DAVID A. BOYD, JR., AND JOHN H. CONLIN

From the Research and Biological Laboratories, Parke, Davis & Company, Detroit, Michigan and the Ypsilanti State Hospital, Ypsilanti, Michigan

Some severe local reactions have been observed following the hypodermic injection of staphylococcus bacteriophage. Such reactions are controlled, to a certain degree, by size of dose and manner of injection, and according to past experience, do not contraindicate the use of staphylococcus bacteriophage. However, the usual appearance of definite local reactions affords one means of studying some of the constituents of staphylococcus bacteriophage. Therefore this study was undertaken to determine the nature of the reaction-producing substance in this preparation.

METHOD

The procedure involved the comparative measurement and evaluation of local reactions following the intradermal injection of 0.1 cc. or the various materials studied. The reactions were measured in millimeters and recorded as "circumscribed" or "diffuse," and also as "very red," "red," or "faint red" in intensity. Independent readings were made by two observers to exclude personal bias. While local reactions are commonly observed following injection of staphylococcus bacteriophage prepared by different laboratories, an effort was made in this study to avoid variation in the materials by using only the ingredients and products of one lot of staphylococcus bacteriophage. To avoid the possible sensitization of test subjects, a new group of individuals was used for each series of injections. When any comparison of reactions was attempted, all the substances under investigation were injected in the same subject at the same time to insure a comparative standard. In the beginning all reactions were read at 24, 48, and 72 hours, but it was observed that the

* This study was made possible through the kindness and coöperation of Dr. George F. Inch, Superintendent of the Ypsilanti State Hospital.

reaction reached the maximum at 24 hours and nothing could be learned from the fading residual. Therefore, in this investigation, the 24-hour reaction was the only one considered.

In order to conduct comparative studies of reactions under different conditions, it was necessary first to determine the reaction effect following the injection of staphylococcus bacteriophage. Fifty subjects were given 0.1 cc. of staphylococcus bacteriophage. Of these forty-seven showed a marked erythema which in some cases was almost inflammatory in character. To this group was also given simultaneously; 0.1 cc. of 1:10 dilution of the same bacteriophage, and forty-four positive results were secured, but the reaction was markedly diminished in size and intensity. Also simultaneous injection of 0.1 cc. of 1:20 dilution of staphylococcus

TABLE 1

	UNDILUTED BACTERIOPHAGE	1:10 DILUTION	1:20 DILUTION
	mm.	*mm.*	*mm.*
1	60 x 70 (very red)	16 x 40	10 x 14
2	45 x 60 (very red)	20 x 35	12 x 13

bacteriophage gave forty-four positive reactions but with a further diminution of the size and intensity of the reaction.

Table 1 illustrates two typical reactions from the series of fifty cases.

In normal individuals the size and intensity of reactions following the intradermal injection of staphylococcus bacteriophage is directly dependent on the concentration of the product.

The heat lability of the reaction-producing factor was then studied. Staphylococcus bacteriophage was boiled for half an hour with reflux condensation to avoid concentration, and the resulting product was given intradermally to fifty subjects in doses of 0.1 cc. Reactions resulted in forty-five cases but the size and intensity was much less than with the untreated phage. Bacteriophage was then subjected to autoclaving, the first portion being heated for 30 minutes at 15 pounds and the second portion, a total of 70 minutes, 30 minutes at 15 pounds, and 40 minutes

22 pounds. The first gave forty-four positive reactions which were much less marked than those of the boiled product. The second portion gave reactions in thirty-one cases, and the reaction in every case was much smaller and less intense than with the portions which had been subjected to less heat.

Table 2 shows three typical reactions from a series of fifty cases.

The reaction-producing substance in staphylococcus bacteriophage is sensitive to heat and the severity of local reaction is diminished in proportion to the degree of heat applied to the product.

TABLE 2

	UNTREATED PHAGE	BOILED PHAGE	AUTOCLAVED PHAGE	
			30 minutes, 15 pounds	30 minutes, 15 pounds 40 minutes, 22 pounds
	mm.	*mm.*	*mm.*	*mm.*
1	45 x 48 (red)	27 x 27 (red)	17 x 18 (red)	10 x 10 (red)
2	47 x 75 (red)	25 x 40 (red)	18 x 20 (red)	10 x 10 (faint)
3	28 x 45 (red)	20 x 30 (red)	12 x 15 (red)	10 x 11

TESTS WITH FRACTIONS OF BACTERIOPHAGE

For the purpose of this study, staphylococcus bacteriophage was considered to consist of (a) *bouillon;* (b) *staphylococcus toxin;* (c) *disintegrated staphylococci* (bacterial protein, endotoxin, and metabolic products); (d) *bacteriophage* (some substance or property that causes lysis).

Bouillon

The local reaction caused by Leibig's bouillon in 0.1 cc. doses was first investigated. The bouillon was given intradermally to a series of fifty individuals in 0.1 cc. doses of a 1:20 and 1:60 dilution, but no positive reactions were observed. The bouillon was then given intradermally in undiluted form to another series of fifty individuals with only one small, faint reaction. The bouillon was used as a control in one hundred and fifty other subjects and failed to cause any local reaction. Fifteen individuals were then selected and given 1 cc. of bouillon hypodermically with no

reaction resulting. Twenty-five new subjects were then given 5 cc. of bouillon hypodermically and all results were equally negative.

Bouillon is not a reaction-producing factor in staphylococcus bacteriophage.

Staphylococcus Toxin

In determining the role of staphylococcus toxin in the production of the local reaction, it was first necessary to separate this substance. This was done by growing *Staphylococcus aureus* in Leibig's bouillon for 48 hours under the same conditions as those followed in the preparation of bacteriophage and filtering the

TABLE 3

TOXIN

	0.1 cc.	1:10 DILUTION	1:20 DILUTION	BOILED ONE-HALF HOUR	AUTOCLAVED	
					30 minutes, 15 pounds	30 minutes, 15 pounds 40 minutes, 22 pounds
	mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
1	42 x 45	20 x 22	15 x 17	24 x 32	12 x 15	7 x 6
2	55 x 65	24 x 25	13 x 17	21 x 22	Negative	Negative
3	42 x 60	25 x 25	17 x 22	14 x 30	10 x 10	6 x 7

culture through a Pasteur filter. The resulting filtrate was a sterile mixture of bouillon and staphylococcus toxin. The absence of staphylococcus bacteriophage was proved by a series of lytic tests. Then a new series of fifty subjects was selected and the same procedure followed as with bacteriophage. The skin reaction from 0.1 cc. intradermal doses of this staphylococcus toxin approached in size and intensity the reaction caused by 0.1 cc. doses of staphylococcus bacteriophage. Dilution of the toxin caused an analogous diminution in the size of the reaction. The heated toxin solution followed the behavior of heated staphylococcus bacteriophage, that is, the more heat applied, the smaller and less intense the skin reaction.

Table 3 records three typical reactions from a series of fifty cases.

Staphylococcus toxin, in reaction-producing properties, behaves in practically the same manner as staphylococcus bacteriophage and is similarly influenced by dilution and by heat.

Disintegrated staphylococci

A 48 hour culture of *Staphylococcus aureus* was centrifuged and the bacteria separated from the broth and toxin. The bacteria were then suspended in a volume of physiological saline equal to

TABLE 4

MATERIAL	PERSONS TESTED	POSITIVE REACTIONS
Undiluted suspension	25	20
1:10 suspension	25	7
1:20 suspension	25	1
Boiled one-half hour	25	20
Autoclaved 30 minutes, 15 pounds	25	16
Autoclaved 30 minutes, 15 pounds; 40 minutes, 22 pounds	25	16

TABLE 5

BACTERIAL SUSPENSION

	0.1 cc.	1:10 DILUTION	1:20 DILUTION	BOILED ONE-HALF HOUR	AUTOCLAVED	
					30 minutes, 15 pounds	30 minutes, 15 pounds 40 minutes, 22 pounds
	mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
1	30 x 30	15 x 15	8 x 8	18 x 18	18 x 18	15 x 19
2	15 x 15	Very slight	Negative	11 x 11	10 x 11	11 x 11
3	Faint	Negative	Negative	Faint	Negative	Negative

the volume of the original culture. The growth was destroyed with 0.2 per cent tricresol. The resulting suspension was then allowed to stand for several days to allow disintegration of the bacteria and to insure sterility. This solution was tested and found to have no lytic principle. A series of twenty-five subjects were then selected and the diluted and heat-treated products of the suspension injected intradermally. The reaction from the suspension was usually a small area which was neither as inflam-

matory nor as definitely outlined as either the bacteriophage or toxin reactions. Dilutions of the suspension gave very few reactions which were usually of small size. Heat seemed to have very little effect on the bacterial protein and heat-treated solutions usually gave reactions on those individuals who reacted to the untreated protein.

The results are indicated in table 4.

Typical reactions from a series of twenty-five cases are indicated in table 5.

Bacterial protein from Staphylococcus aureus is a positive but minor factor in the production of local reactions.

Staphylococcus bacteriophage

It is impossible by the use of heat to destroy the lytic principle in staphylococcus bacteriophage without also affecting the toxin.

TABLE 6

	BACTERIOPHAGE			CONTROL		CONTROL BOUILLON
	0.2 per cent tricresol	0.5 per cent phenol	Untreated	0.2 per cent tricresol bouillon	0.5 per cent phenol bouillon	
	mm.	*mm.*	*mm.*			
1	40 x 50 (red)	40 x 50 (red)	30 x 55 (red)	Negative	Negative	Negative
2	35 x 47 (red)	35 x 48 (red)	30 x 50 (red)	Negative	Negative	Negative
3	47 x 60 (red)	40 x 65 (red)	45 x 65 (red)	Negative	Negative	Negative

After a series of tests, it was determined that the lytic principle was destroyed by 0.2 per cent tricresol and also by 0.5 per cent phenol.

One lot of staphylococcus bacteriophage was divided into three parts. To the first portion was added tricresol (0.2 per cent); to the second portion was added phenol (0.5 per cent); the third portion was used as the control. The results of tests proved that lysis was absent in the first and second, but present and active in the third. Another series of twenty-five individuals was given 0.1 cc. doses of the three portions with control doses of 0.2 per cent tricresol bouillon, 0.5 per cent phenol bouillon, and unpreserved bouillon. The three phage solutions caused reactions

approximately equal in size and intensity. The controls were consistently negative.

Table 6 shows typical results from a series of twenty-five cases.

The chemical destruction of the lytic principle in staphylococcus bacteriophage does not diminish the size and intensity of the skin reaction.

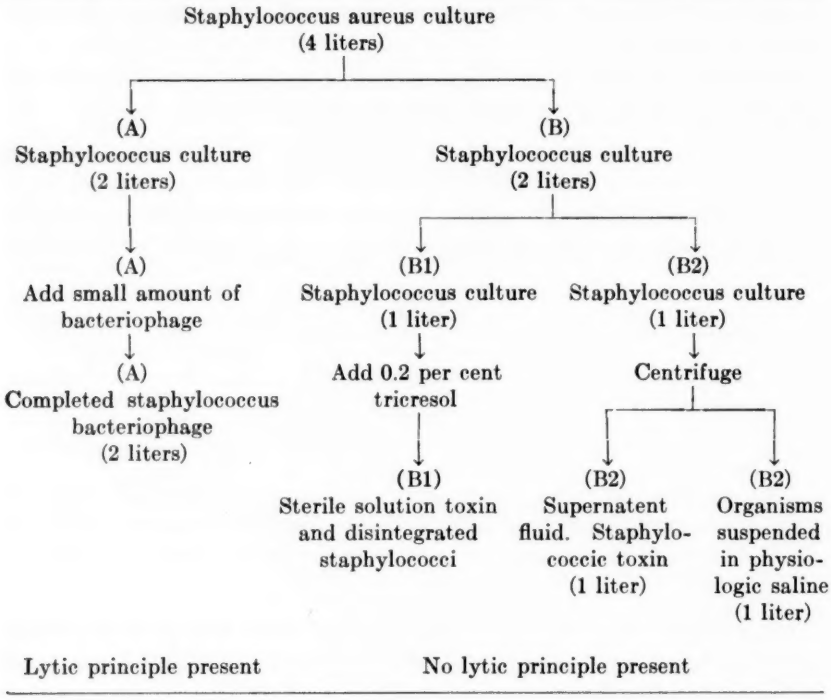

CHART 1

DIRECT COMPARISON OF REACTION-PRODUCING CONSTITUENTS

Staphylococcus aureus was planted into 4 liters of Leibig's bouillon. At the end of 48 hours the culture was divided into two equal parts (A and B). The flask "A" was added to a small amount of staphylococcus bacteriophage of known activity and allowed to undergo complete lysis, with the consequent production of 2 liters of ordinary staphylococcus bacteriophage. When

flask "A" began to clear, culture "B" was divided into two equal parts (B1 and B2). To B1 was added 0.2 per cent tricresol to destroy growth, with the subsequent production of a bouillon solution of staphylococcus toxin and disintegrated staphylococci in a concentration equal to that in the completed staphylococcus bacteriophage. At the same time, culture B2 was centrifuged and the organisms thrown down. The supernatant fluid was removed and passed through a Pasteur filter to insure sterility. This fluid (1 liter) represented bouillon solution of staphylococcus toxin in a concentration equal to that in the completed staphylococcus bacteriophage. The organisms which had been thrown down were suspended in a liter of physiological saline, making a staphylococcus suspension of a concentration equal to that of

TABLE 7

	COMPLETED BACTERIOPHAGE (1)	STAPHYLOCOCCI TOXIN AND DISINTEGRATED STAPHYLOCOCCI (2)	STAPHYLOCOCCUS TOXIN (3)	DISINTEGRATED STAPHYLOCOCCI (4)	BOUILLON CONTROL
	mm.	*mm.*	*mm.*	*mm.*	
1	55 x 80 (red)	60 x 75 (red)	55 x 60 (red)	30 x 35 (red)	Negative
2	45 x 80 (red)	60 x 65 (red)	45 x 55 (red)	30 x 35 (faint red)	Negative
3	70 x 105 (red)	80 x 95 (red)	65 x 90 (red)	33 x 40 (red)	Negative
4	85 x 115 (red)	71 x 105 (red)	60 x 115 (red)	5 x 5 (faint red)	Negative
5	51 x 85 (red)	50 x 60 (red)	44 x 80 (red)	20 x 24 (red)	Negative

disintegrated staphylococci in the completed staphylococcus bacteriophage. Further growth was destroyed by 0.2 per cent tricresol.

Repeated tests for lysis showed that the lytic principle was absent in every fraction except in the staphylococcus bacteriophage. In the latter it was present and active. (See chart 1.)

This procedure gave four fractions: the concentration of each active principle was equivalent to its concentration in the completed staphylococcus bacteriophage. It was then possible to judge the reaction-producing properties of each component in comparison to each other and in comparison to the completed staphylococcus bacteriophage. Intradermal injections (0.1 cc. doses) were given to a new series of twenty-five individuals. Typical reactions from the series are shown in table 7.

From the above results it can be seen that the skin reaction of completed staphylococcus bacteriophage (1) is approximately equal to the reaction of combined staphylococcus toxin and disintegrated staphylococci; (2) the reaction to completed bacteriophage is approximately the sum of the reactions to staphylococcus toxin and disintegrated staphylococci; (3) the reaction of staphylococcus toxin approaches in magnitude and intensity the reaction of the completed bacteriophage; (4) the skin response to disintegrated staphylococci is small and slight in comparison to that in the case of staphylococcus toxin or completed bacteriophage.

The reaction caused by the intradermal injection of staphylococcus bacteriophage is practically equal to that produced by an equal concentration of staphylococcus toxin and disintegrated staphylococci from the same lot of product. The reaction is largely due to staphylococcus toxin but is in some degree dependent upon the presence of disintegrated staphylococci. The presence or absence of the lytic principle does not influence the frequency or degree of reaction.

SUMMARY

The present study has included the use of 425 individual test subjects who received 1625 intradermal injections, and forty individuals who were given hypodermic injections.

The results clearly indicate that the major reaction-producing factor in staphylococcus bacteriophage is the staphylococcus toxin present.

In consideration of these findings, the question arises as to whether the therapeutic value ascribed to staphylococcus bacteriophage when used hypodermically, is due in some measure to the presence of staphylococcus toxin.

REFERENCES

(1) Borthwick, G. R.: Experimental observations on the toxic effects of staphylococcal filtrates introduced internally in laboratory animals. Brit. Jour. Exper. Path., **14:** 236–240. 1933.

(2) Bryce, L. M. and Burnet, F. M.: Natural immunity to staphylococcal toxin. Jour. Path. and Bact., **35:** 183–191. 1932.

(3) Burky, E. L.: Effect of intradermal and intravenous injections of toxic staphylococcus filtrates in rabbits of varying ages. Jour. Allergy, **3:** 438–441. 1932.

(4) BURNET, F. M.: Flocculation reaction with staphylococcal toxin. Jour. Path. and Bact., **34:** 759–769. 1931.

(5) DOLMAN, C. E.: Pathogenic and antigenic properties of staphylococcus toxin. Can. Pub. Health Jour., **23:** 125–132. 1932.

(6) DOLMAN, C. E.: Treatment of localized staphylococcus infections with staphylococcus toxoid. Jour. Am. Med. Assn., **100:** 1007–1010. 1933.

(7) GROSS, H.: Das toxin des Staphylococcus pyogenes aureus. Klin. Wchnschr, **11:** 1473–1475. 1932.

(8) PANTON, P. N., AND VALENTINE, F. C. O.: Staphylococcal toxin. The Lancet, **1:** 506–508. 1932.

(9) PARISH, H. J. AND CLARK, W. H. M.: Staphylococcal toxin and antitoxin. Jour. Path. and Bact., **35:** 251–258. 1932.

BLOOD IODINE STUDIES

IV. The Clinical Determination of Iodine in Blood, Urine, and Feces

FRANCIS J. PHILLIPS and GEORGE M. CURTIS

From the Departments of Medical and Surgical Research, and of Surgery, of The Ohio State University, Columbus, Ohio

The interdependence of iodine and normal thyroid function is securely established. Extensive clinical studies are clarifying the significance of the blood iodine.[6, 17, 22] Sturm's discovery,[22] that the blood iodine is increased in hyperthyroidism, has been amply confirmed. There is an increased loss of urinary iodine in patients with hyperthyroidism.[7] The relation of iodine metabolism to toxic goiter is receiving extensive attention. As a consequence the blood iodine, the urinary excretion of iodine, the iodine of the feces and the loss of iodine in the perspiration are assuming an increasing import in clinical investigation. As these investigations are becoming more widely known and appreciated, adequate clinical methods for the biochemical determination of iodine are being sought.

Quantitative methods for the determination of the minute amount of iodine normally present in living things have long been in use. These methods, however, have, in the past, proved to be successful only in the hands of a few experienced chemists. In fact, the early results of Chatin,[3, 4] published between 1850 and 1876, were widely doubted and later forgotten. Contemporary chemists could not verify them. von Fellenberg's[10] extensive and convincing studies have given them a belated confirmation.

Bourcet,[2] Blum and Grützner,[1] Hunter,[11] Kendall,[14] von Fellenberg, Leitch and Henderson,[16] Remington,[20] McClendon,[18] Turner,[21] Veil and Sturm,[22] Lunde and Closs,[17] Davis and Curtis,[9] Karns[12, 13] and von Kolnitz,[15] have contributed to the development of methods for the determination of iodine in biological

substances. Recent progress is largely the result of von Fellenberg's contributions.

The primary purpose in making this particular investigation was to develop a method for use in the clinical laboratory. The method here presented is based upon the fundamental principles described by von Fellenberg. It makes possible the extensive clinical investigation of iodine metabolism. We have used it in investigating the blood iodine[6] and in other studies.[5, 7, 8] The volatility of iodine and the dissociability of its compounds make the analyses of such small amounts as are present in blood extremely difficult. Therefore, best results are obtained when the procedure is followed meticulously and is carried out in the minimum amount of time.

BLOOD

Blood is analyzed in 10 cc. duplicate samples. While carefully rotating a thoroughly cleaned 6 cm. nickel crucible containing an accurately measured portion of whole oxalated blood, 10 cc. of a saturated aqueous solution* of *iodine free* potassium hydroxide is added. The resulting dark mass of blood and KOH is boiled on a hot plate, or over a Bunsen flame, until the proteins are well hydrolyzed. When the mass has condensed to the point that it no longer foams vigorously, hydrolysis is usually complete. The crucible containing the homogenous mass is then placed in the muffle furnace set at 400°C. During this time and during the succeeding heating the crucible must be carefully watched in order to detect and control any excessive bubbling and frothing that occasionally may occur. An automatic temperature controller is necessary to maintain the muffle furnace constantly at the above mentioned temperature. After heating for one half hour the crucible is removed, cooled, and the mass thoroughly moistened with *iodine free* distilled water. The crusting on the walls of the crucible is well rinsed down. The water is best evaporated over a Bunsen burner. The crucible is then replaced in the muffle furnace. A slow stream of oxygen is admitted from a large cylinder to the combustion chamber of the furnace. The cooling and moistening process is repeated at twenty minute intervals. This procedure must be repeated until the charred mass is completely oxidized. When completely oxidized the original mass has a grayish appearance.

---

* We are indebted to Dr. D. Roy McCullagh of the Cleveland Clinic for his recent suggestion as to the use of large amounts of KOH in hydrolyzing the blood before it is ashed.

The water soluble salts are now extracted from this completely oxidized mass with three 15 cc. portions of iodine free distilled water. The solution is prevented from creeping over the edge of the crucible by greasing the lip with a thick vaseline. Each extract is filtered through the same Whatman No. 44 filter paper. This grade of filter paper, 9 cm. in diameter, has been found adequate. The filtrates are collected in another thoroughly cleaned nickel crucible. A nickel crucible is used in this stage because it will better withstand the later scraping and kneading of the dried salts. The filter paper is *always* allowed to drain between extractions. The transfer of the extract *must be done quantitatively*. After the last transfer has drained down, the inside of the funnel is sprayed with a few cubic centimeters of iodine free distilled water to insure complete solution and filtration of any remaining salts. The filter paper containing the insoluble oxides, and salts, is discarded. Repeated analyses of these filter papers, and of the contained oxides and salts, have shown that no appreciable amount of iodine remains in the filter paper after this thorough washing.

To make possible the alcoholic extraction of the iodine salts from the salt mixture, the combined distilled water extract from the filtrations is evaporated nearly to dryness, preferably on a steam bath, or in any way that does not cause spattering. The crucible is then removed and the remaining liquid carefully evaporated to a semi-viscid consistency over the low flame of a micro burner. The crucible should be constantly rotated during this process. This rotation minimizes spattering at the beginning of the evaporation process. At the end it facilitates the formation of smaller crystals. The presence of small crystals is necessary for the complete extraction of the iodine salts with alcohol. The optimum amount of evaporation is readily learned from experience. The point at which the solution begins to show crystallization, as evidenced by the formation of large bubbles, indicates an adequate amount of evaporation. The rotation must be kept up after the crucible has been removed from the flame and until the salts have completely crystallized into a firm grayish mass. This crystallization will occur at room temperature.

The iodine salts are now extracted from the mass of crystallized salts with alcohol. *Iodine free* ethyl alcohol is used. Ninety-five per cent concentration has been found to be optimum for the most complete extraction. Since the iodine salts are readily soluble in ethyl alcohol, they are quantitatively extracted with three 10 cc. portions. Only a minimum of other salts is brought over in the extract. It is important to minimize the amount of other salts in the extract because they may interfere with the titration. The original mass of salts, which should now be a thick homogenous white paste, is carefully kneaded in the alcohol with a chisel shaped metal rod. The entire inside of the crucible should be well scraped to insure complete extraction of any adhering particles. Each alcoholic extract is then quantitatively transferred to a 125 cc. Erlenmeyer flask.

This cumulative alcoholic extract is now evaporated to dryness on a steam bath. If the alcohol is not all boiled off the final titration color may be confusing. The crystallized salt mixture is then redissolved in *iodine free* distilled water. For the first addition 25 cc. are sufficient. Two drops of a 0.0007 molar methyl orange solution are now added. The pH is adjusted to about 4.0 by titrating the solution to a faint pink with 0.05 molar hydrochloric acid. Two cc. of a *freshly prepared iodine free* chlorine saturated water are now added. The pink color disappears on the addition of the chlorine water. The solution is now boiled gently over a Bunsen flame. The flask should be gently rotated to prevent undue bubbling and spattering. Boiling off about 45 cc. of water has been found adequate to insure complete removal of the excess chlorine gas. The original 25 cc. of water in the flask is boiled down to about 5 cc. Then 25 cc. more of iodine-free distilled water are added and boiled down to 5 cc. Before titration the concentrate must be cooled to about 25°C. The blue color of the starch iodine reaction does not appear unless the solution is well cooled. One small crystal of potassium iodide is now added. Too great an excess of potassium iodide causes a violet color to form. This color makes a recognition of the end point difficult. Six drops of a freshly prepared solution of soluble starch, (about 0.5 per cent), are now added. If iodine is present the characteristic blue color, commensurate in density to the amount of iodine that is free in the solution will appear within two minutes. The free iodine is then titrated with 0.001 normal sodium thiosulphate solution, using an especially devised micro-reservoir burette.[19]

URINE

Urine is analyzed in 25 cc. duplicate samples. While the crucible is being carefully rotated 1.5 cc. of saturated KOH are added. The KOH prevents loss of iodine when thoroughly mixed with the urine. The mixture is then carefully boiled to dryness. This requires about one-half hour. The resulting mass is heated in the muffle furnace for about twenty minutes at 400°C., without supplemental oxygen. The crucible is removed from the furnace, cooled, and the ash moistened with *iodine free* distilled water. It is then heated again in the furnace at 400°C., with supplemental oxygen, as in ashing blood. In one-half hour a nearly white mass of salts remains. The remainder of the process is identical with that used for blood.

FECES

The principles employed are identical with those as given for blood. Since there is a greater amount of organic matter present, it is necessary to add more of the saturated KOH solution. Therefore, 1.5 cc. per gram are used in order to obtain adequate hydrolysis. If the stool has a high fat content, alcoholic hydrolysis may be necessary. Before this is employed the mass should be well heated over a Bunsen flame until it is of even consistency. The crucible is

then placed on the steam bath and 25 cc. of 95 per cent ethyl alcohol are added. One addition is usually sufficient, although more may be necessary. The muffle furnace oxidation is carried out as with blood. This is not difficult if the material is well hydrolyzed and, consequently, homogenous. For extraction, the water and alcohol increments are increased proportionately to the amount of KOH employed. Titration is the same.

THYROID GLAND

Analyses may be accomplished by using the same procedure and the same proportion of reagents as for blood.

MILK

Analyses of the iodine content of milk are identical with those used for blood, except that alcoholic hydrolysis is employed as described under the method for feces.

WATER

Water is analyzed in the same manner as urine. Less time is necessary because of the minute amount of organic matter normally present.

REAGENTS

Potassium Hydroxide, saturated aqueous solution. This is made by saturating 1000 cc. of *iodine free* distilled water with "Kahlbaum" KOH.

Sodium Thiosulphate, 0.1 normal. Dissolve 24.832 gm. Merck's Blue Label, or P-W-R, crystalline salt in *iodine free* distilled water and make up to 1000 cc. This reagent should be kept in a dark bottle that is sterile[9] as well as chemically clean. If the reagent is stored in a cool place the normality changes very slightly in months. The reagent is checked against the KIO_3-HIO_3 standard every two months. The 0.001 normal for the titration is made fresh each day by diluting 1 cc. of the 0.1 normal to 100 cc. in a volumetric flask.

Potassium Di-iodate, 0.1 normal. Dissolve 3.2496 gms. of KIO_3-HIO_3 in *iodine free* distilled water and dilute to 1000 cc. This reagent is used as the standard check for the sodium thiosulphate.

Ethyl Alcohol, 95 per cent. Made by diluting absolute alcohol with *iodine free* distilled water to 95 per cent.

Methyl Orange, 0.0007 molar. Dissolve 0.020 gm. of the sodium salt of dimethylamino-azo-benzene-sulphonic acid in 100 cc. of *iodine free* distilled water.

Chlorine Water. A saturated solution is made by bubbling chlorine gas from a cylinder into a convenient volume of *iodine free* distilled water until the solution is yellow. Chlorine gas manufactured by the Matheson Alkali Co., of East Rutherford, N. J., has been found to be iodine free.

Potassium Iodide. Merck's Blue Label, or P-W-R, is satisfactory.

Starch Solution, 0.5 per cent. One-half gram of soluble starch, prepared

according to Lintner, is made into a thin paste and added to 100 cc. of boiling *iodine free* distilled water. The boiling is continued for one minute after the addition of the starch paste or until the solution is clear. If the solution is not clear then the starch is not adequately dissolved, and therefore unsatisfactory.

Hydrochloric Acid, 0.05 normal. Merck's Blue Label is satisfactory. This reagent need be adjusted only approximately to the specified normality.

COMMENT

The chemical principles involved in the procedure are as follows: The oxidation of the blood in the presence of an excess of KOH

TABLE 1

DETERMINATIONS OF IODINE IN BEEF BLOOD MADE UPON THE SAME SAMPLE

FIRST DAY	SECOND DAY	THIRD DAY
gamma per 100 cc.	*gamma per 100 cc.*	*gamma per 100 cc.*
14.4	12.3	16.1
14.8	14.6	15.2
14.4	14.6	16.3
14.6	15.7	16.5
14.4	14.4	16.7
15.8	13.0	12.5
12.5	15.3	16.3
13.1	13.4	16.9
		16.1
		15.6
Average....14.3	14.2	15.8

Grand average for twenty-six samples.....14.8

facilitates the formation of KI and possibly KIO_3 as rapidly as iodine is liberated from such other combinations as may exist. All the iodine present is eventually oxidized to iodate by means of chlorine gas. The oxidation occurs as follows:

$$2\ I^- + Cl_2 \rightarrow I_2 + 2\ Cl^-$$

$$I_2 + 5\ Cl_2 + 6\ HOH \rightarrow 2\ IO_3^- + 10\ Cl^- + 12H^+$$

When the iodate is reduced to iodine, by means of KI in an acid solution, the original value of the iodine is increased six-

fold.[9, 23, 24] This reaction takes place rapidly and quantitatively at a pH below about 3.

$$IO_3^- + 5I^- + 6\ H^+ \rightarrow 3I_2 + 3\ HOH$$

Twenty-six analyses of the same sample of beef's blood from the slaughter-house, were made in three groups on consecutive days. The lowest value was 12.3, the highest 16.9, with an average of 14.8 gamma per cent for the entire series. The individual analyses are presented in table 1. When known additions of $KIO_3 - HIO_3$ were made to the blood samples the recovery varied from 80 to 94 per cent.

CALCULATION

$$1 \text{ cc. of } 0.001 \text{ N } Na_2S_2O_3 = \frac{126.993}{1{,}000 \times 1{,}000 \times 6} = 21.15 \text{ Gamma* of iodine}$$

$$(X) \text{ cc. of } 0.001 \text{ N } Na_2S_2O_3 \times 21.15 \times \frac{100}{n} \text{ Gamma of iodine per 100 cc. (or per 100 gm.)}$$

n = cc. of specimen, or gm. of specimen

REFERENCES

(1) Blum, F., and Grützner, R.: Studien zur Physiologie der Schilddrüse. Ztschr. f. physiol. Chem., **85:** 429–470. 1913.

(2) Bourcet, P.: Recherche et dosage colorimétrique de petites quantitées d'iode dans les matières organiques. Compt. rend. Acad., Sc., **128:** 1120. 1899.

(3) Chatin, Ad.: Existence de l'iode dans les plantes d'eau douce. Compt. rend. Acad., Sc., **30:** 352. 1850.

(4) Chatin, Ad.: Des causes d'insuccès, dans la recherche des minimes quantitées d'iode. Compt. rend. Acad., Sc., **82:** 128. 1876.

(5) Curtis, G. M.: Iodine metabolism in toxic goiter. In press.

(6) Curtis, G. M., Davis, C. B., and Phillips, F. J.: Significance of the iodine content of human blood. Jour. Am. Med. Assn., **101:** 901–904. 1933.

(7) Curtis, G. M., and Phillips, F. J.: The urinary excretion of iodine in toxic goiter. Jour. Am. Med. Assn., **101:** 1992–1993. 1933.

(8) Curtis, G. M., and Phillips, F. J.: Newer aspects of the management of hyperthyroidism. Ohio State Med. Jour., **30:** 149–155. 1934.

* Gamma = microgram.

(9) DAVIS, C. B., AND CURTIS, G. M.: Blood iodine studies. Jour. Lab., Clin. Med., **18:** 24–29. 1932.

(10) VON FELLENBERG, TH.: Das Vorkommen, der Kreislauf und der Stoffwechsel des Jods. Ergebn. d. Physiol., **25:** 176–363. 1926.

(11) HUNTER, A.: The determination of small quantities of iodine, with special reference to the iodine content of the thyroid gland. Jour. Biol. Chem., **7:** 321–349. 1910.

(12) KARNS, G. M.: Ashing apparatus for samples containing traces of iodine. Ind., Eng. Chem. Analyt. Ed., **4:** 299–300. 1932.

(13) KARNS, G. M.: Collecting train for recovering traces of iodine from ashed samples. Ind., Eng. Chem. Analyt. Ed., **4:** 375–377. 1932.

(14) KENDALL, E. C., AND RICHARDSON, F. S.: Determination of iodine in blood and in animal tissues. Jour. Biol. Chem., **43:** 161–170. 1920.

(15) VON KOLNITZ, H., AND REMINGTON, R. E.: A simplified Karns technic for the microestimation of iodine. Ind., Eng. Chem. Analyt. Ed., **5:** 38–39. 1933.

(16) LEITCH, I., AND HENDERSON, J. M.: The estimation of iodine in food stuffs and body fluids. Biochem. Jour., **20:** 1003–1007. 1926.

(17) LUNDE, G., AND CLOSS, K.: Om bestemmelse av blodjodspeilet. Norsk. Mag. f. laegevedensk., **89:** 500–506. 1928.

(18) MCCLENDON, J. F.: The determination of traces of iodine. I. Jour. Am. Chem. Soc., **50:** 1093–1099. 1928.

(19) PHILLIPS, F. J., AND CURTIS, G. M.: A simple reservoir burette for making microtitrations. In press.

(20) REMINGTON, R. E., MCCLENDON, J. F., AND VON KOLNITZ, H.: The determination of traces of iodine. Jour. Am. Chem. Soc., **53:** 1245–1249. 1931.

(21) TURNER, R. G.: A micro-colorimetric method for the quantitative estimation of iodine in blood. Jour. Biol. Chem., **88:** 497–511. 1930.

(22) VEIL, W. H., AND STURM, A.: Beiträge zur Kenntnis des Jodstoffwechsels. Deut. Arch. f. klin. Med., **147:** 166–223. 1925.

(23) WINKLER, L. W.: Bestimmung des Broms und Jods in Gegenwart von Chloriden. Ztsch. f. angew. Chem., **28:** 477–480. 1915.

(24) WINKLER, L. W.: Der Jodid- und Jodationgehalt des Meerwassers. Ztsch. f. angew. Chem., **29:** 205–207. 1916.

THE ACCURACY OF COMMON HEMOGLOBIN METHODS

HOWARD L. ALT*

Department of Medicine, Northwestern University Medical School, Chicago, Illinois

A survey of eighteen representative hospitals in this community was made recently to find out what methods of hemoglobin determination were in common use. Of the hospitals investigated, nine used the Tallquist method, five the Sahli, and four the Newcomer. In institutions where the Tallquist was used routinely, the Sahli or Dare method was performed in cases of anemia. Of all the instruments in use, only one had been calibrated against a more accurate method. The hemoglobin was reported in per cent, while in a few instances it was also recorded in grams per 100 cc. or in Sahli units. There was no uniform opinion among laboratory workers as to the relative accuracy of the instruments in use. A review of the literature did not reveal consistent and exact information concerning the comparative accuracy of the Tallquist, Dare, Sahli, and Newcomer instruments. The present study was undertaken to determine the average per cent error of these methods when performed under optimum conditions.

EXPERIMENTAL

In determining the accuracy of the common hemoglobin instruments, the calculation of the blood hemoglobin from the blood iron content (hemoglobin = 0.335 per cent iron) was used as a standard of comparison. This method[4,7] has been shown to check closely with the oxygen capacity method, and has the advantage of being much simpler. Oxalated venous blood obtained from students, nurses and ambulatory patients was used for testing.

* With the technical assistance of Elizabeth Jane.

Duplicate determinations of the blood iron by the modified Wong[12] method and of the hemoglobin by the Newcomer, Sahli, and Dare methods were made on thirty-five samples of blood. An average of each two determinations was used for comparative purposes. The Tallquist method was included at first, but since blood hemoglobin values between 60 and 100 per cent could not be read with any degree of certainty, it was discarded as being inadequate for routine clinical work.

Iron content of blood

The blood iron was determined by the modified Wong method. It consists in the digestion of 0.5 cc. blood with concentrated sulphuric acid and potassium persulphate without heating, dilution,* precipitation of the proteins with tungstic acid, development of a color with potassium thiocyanate, and reading colorimetrically against a standard. All water was distilled in glass. Baker's C. P. potassium persulphate was used as this preparation gave no blank test for iron. Acid-washed filter paper was used throughout. This method was found to be simple, rapid and accurate, and is well adapted for use in the blood chemistry laboratory.

Newcomer

Determinations by the Newcomer method were made on a DuBoscq colorimeter fitted with a standard yellow disc and a blue glass filter (Bausch and Lomb). Artificial light was used for illumination. Two dilution pipettes were used and were found to check uniformly. Exactly thirty minutes were allowed between the dilution (1–500) and the colorimetric reading. No corrections were made for development of color.

Sahli

The Sahli determinations were performed with a Hellige type of instrument which contains two colored glass prisms in apposition to the diluting tube. Two pipettes and two cylindrical diluting tubes (used for all the tests) gave equivalent results. In the test, after adding blood to the small amount of N/10 HCl, exactly 10 minutes were allowed before diluting to the proper color. Since the color of the acid hematin continues to develop after this time, it is necessary to calibrate the instrument to a uniform length of time. In reading the end

* A precipitate that sometimes occurred at this point could be prevented if the blood and sulphuric acid were gently mixed and allowed to stand for two minutes instead of whirling as recommended by Wong. Determinations were more difficult to check when the precipitate occurred after water was added to the digestate.

TABLE 1

Comparison of Common Hemoglobin Methods

Per cent variation from the blood iron method

Number	Blood iron		Newcomer		Sahli		Dare	
	Iron	Calculated hemoglobin	Factor: 1.047*		14.1 grams—100 per cent†		17.76 grams—100 per cent**	
			Hemoglobin	Variation	Hemoglobin	Variation	Hemoglobin	Variation
	mgm. per 100 cc.	*grams per 100 cc.*	*grams per 100 cc.*	*per cent*	*grams per 100 cc.*	*per cent*	*grams per 100 cc.*	*per cent*
1	40.60	12.1	11.7	3.3	11.7	3.3	12.8	5.8
2	42.64	12.7	13.6	7.1	13.2	3.9	13.0	2.4
3	43.29	12.9	13.2	2.3	13.2	2.3	13.3	3.1
4	43.38	12.9	13.5	4.7	12.7	1.5	13.3	3.1
5	43.86	13.1	14.6	11.5	14.0	6.9	14.2	8.4
6	44.94	13.4	12.0	10.4	13.1	2.2	13.0	3.0
7	44.98	13.4	14.1	5.2	14.1	5.2	14.9	11.2
8	45.40	13.6	14.1	3.7	13.7	0.7	14.9	9.6
9	45.55	13.6	13.5	0.7	13.2	2.9	13.7	0.7
10	45.55	13.6	14.1	3.7	14.0	2.9	13.0	4.4
11	45.56	13.6	12.9	5.1	12.8	5.9	13.7	0.7
12	45.66	13.6	12.8	5.9	13.2	2.9	13.7	0.7
13	46.30	13.8	13.6	1.4	13.7	0.7	14.0	1.4
14	47.50	14.2	13.8	2.8	13.7	3.5	14.6	2.8
15	49.02	14.6	15.1	3.4	14.5	0.7	14.2	2.7
16	49.53	14.7	15.0	2.0	14.9	1.4	16.5	12.3
17	49.54	14.8	14.6	1.4	15.4	4.1	14.0	5.4
18	50.25	15.0	15.4	2.7	15.1	0.7	15.1	0.7
19	50.50	15.1	14.2	6.0	15.2	0.7	14.9	1.3
20	50.76	15.1	15.8	4.6	15.9	5.3	14.4	4.6
21	51.55	15.4	15.0	2.6	15.4	0.0	14.6	5.2
22	51.68	15.4	15.8	2.6	15.1	1.9	15.4	0.0
23	52.63	15.7	15.9	1.3	14.8	5.7	14.9	5.1
24	52.70	15.7	15.4	1.9	15.2	3.2	15.6	0.6
25	52.90	15.8	15.9	0.6	16.1	1.9	15.1	4.4
26	53.04	15.8	14.9	5.7	15.5	1.9	15.6	1.3
27	54.60	16.3	16.0	1.8	16.1	1.2	15.4	5.5
28	54.70	16.3	15.7	3.7	15.4	5.5	16.5	1.2
29	54.79	16.3	16.1	1.2	16.3	0.0	17.0	4.3
30	55.09	16.4	16.7	1.8	15.8	3.7	14.6	11.0
31	55.25	16.5	16.6	0.6	16.4	0.6	16.0	3.0
32	55.37	16.5	15.9	3.6	16.8	1.8	16.7	1.2
33	55.55	16.6	16.7	0.6	16.2	2.4	16.9	1.8
34	57.81	17.2	16.9	1.7	18.3	6.4	17.6	2.3
35	59.88	17.9	17.8	0.6	18.0	0.6	17.0	5.0
Average per cent variation.....				3.4		2.7		3.9

* Calibration by oxygen capacity method, factor : 1.039.

† Commercial calibration, 17.0 grams — 100 per cent.

** Commercial calibration, not known for this instrument.

point, the instrument was held at arm's length and rotated slightly until the color in the center of the diluting tube merged directly with the color of one of the yellow prisms. Daylight was used as a source of illumination. The intensity of the daylight seemed to make very little difference, although the readings were about 2 to 3 per cent higher by artificial light.

Dare

The Dare instrument used was fitted with a lamp and battery for illumination. Each determination consisted of an average of three readings on one pipette of blood. Attention has been called to the marked discrepancies in the width of the spaces between the glass plates of the pipettes,[6] but the difference between the two pipettes used in these experiments was negligible.

The same set of hemoglobin values was used for calibration of each instrument and also for determining the average per cent variation from the blood iron method. Determinations of the total blood iron and the hemoglobin by the Newcomer method were recorded in grams hemoglobin per 100 cc. while the Sahli and Dare readings were first recorded in per cent. The thirty-five average values were added for each of the four methods. Using the iron value as a standard, an average correction factor was calculated for the Newcomer method, and the grams hemoglobin equivalent to 100 per cent calculated for the Sahli and Dare methods. All figures were then converted to grams hemoglobin per 100 cc. according to these calibrations. These results are recorded in table 1 with their individual and average per cent variation from the blood iron method.

TABLE 2

VARIATION FROM BLOOD IRON IN THIRTY-FIVE HEMOGLOBIN DETERMINATIONS

METHOD	DETERMINATIONS LESS THAN 6 PER CENT	DETERMINATIONS LESS THAN 3 PER CENT
	per cent	*per cent*
Newcomer	89	54
Sahli	94	63
Dare	86	46

The average variation from the blood iron method was 3.4 per cent with the Newcomer, 2.7 per cent with the Sahli, and 3.9 per cent with the Dare method. Table 2 shows the propor-

tion of results with less than 6 per cent and less than 3 per cent variation. The Sahli instrument showed the greatest accuracy of the three when compared with the blood iron method.

In methods involving color matching, the personal equation may influence the accuracy of the results. In order to test this, each of two observers made a single determination on each of ten samples of blood, and the average difference of the two readings was calculated. One observer then made duplicate determinations on another group of ten blood specimens for comparison. Table 3 shows that two observers can check each other's determinations about as well as one observer can check his own. The larger error for the Dare method obtained by a

TABLE 3

AVERAGE DIFFERENCE BETWEEN TWO DETERMINATIONS EACH ON TEN BLOOD SPECIMENS

METHOD	TWO OBSERVERS	ONE OBSERVER
	per cent	*per cent*
Newcomer	0.8	0.6
Sahli	1.3	0.4
Dare	3.6	5.1

single observer may be related to eye fatigue in matching the colors. In general, it was possible to check results with greater accuracy with the Newcomer and Sahli than with the Dare instrument. Although the Newcomer instrument can usually be read within 1 to 2 per cent error, there were occasional blood specimens that had variations up to 10 per cent from the blood iron method, in spite of the fact that both blood iron and Newcomer determinations were rechecked several times. Wintrobe[10] has also noted a similar discrepancy when comparing the Newcomer with the oxygen capacity method.

The results obtained represent the accuracy of common instruments when measuring hemoglobin values within the normal range.* It was our special purpose to find out the accuracy

* Normal range of blood hemoglobin:[11] Males—14 to 18 grams per 100 cc. Females—12–17 grams per 100 cc.

within this range, in order to know what significance to attach to variations in the normal and slightly subnormal levels. To check the accuracy of these methods for anemic blood specimens, series of determinations were made before and after diluting samples of blood with an equal amount of normal saline solution. The diluted blood gave the expected results by the Newcomer and Sahli methods, whereas by the Dare method the results were about 20 per cent too high. Consequently, if the Dare is calibrated for anemic blood specimens, the values obtained for normal blood will be too low. Such a relationship is shown in the figures given by Brown and Roth,[1] who also found the instrument to be quite accurate when measuring lower ranges of hemoglobin values.

DISCUSSION

In considering the accuracy of the hemoglobin determination in the average hospital laboratory, it seems that there is still need for improvement. The Tallquist instrument gives results that are too gross for careful clinical work. Other common methods that are not standardized are subject to considerable error. For example, different Newcomer instruments in the past have shown decided variations in calibration. The marked errors in the commercial calibration of Sahli instruments containing colored glass standards have been pointed out by Cullen.[2] Our results indicate that the Newcomer and Sahli instruments with glass standards are both satisfactory for clinical work when they are properly calibrated and the tests carefully performed. The Sahli method in our hands was found to be somewhat more accurate than the Newcomer method. The Dare method was less satisfactory because of (1) difficulty in checking determinations, (2) the degree of error when measuring values in the normal range, and (3) the variation in calibration at different hemoglobin levels.

Other simple methods for the determination of blood hemoglobin deserve greater trial. Recently, Karshan and Freeman[7] have recommended the use of the acid hematin method of Cohen and Smith. It is shown that the simple iron method of Wong can be substituted for the oxygen capacity method in the prepa-

ration of the standard acid hematin. Osgood and Haskins[5,8] have reported accurate results using a standard inorganic solution to be read colorimetrically or in the Sahli apparatus against an unknown acid hematin solution. It has the drawback of requiring a correction for the temperature of the standard. Sanford and Sheard[9] have obtained good results with the use of a photoelometer. The chief objection to this method is its expense. Results reported with the Haden-Hausser[3] method have been promising. It consists in the comparison of acid hematin with a colored glass standard. Of most importance in adopting any method for the determination of hemoglobin is knowledge as to the accuracy of the results obtained.

The desirability of expressing the hemoglobin content of the blood in grams per 100 cc. has been emphasized by many authors. It is evident that an expression in absolute figures becomes significant only when the method reaches a relative degree of accuracy. Such accuracy is obtainable with some of the common methods of hemoglobin determination.

SUMMARY

(1) The Newcomer, Sahli, and Dare methods of hemoglobin determination were calibrated by comparison with the blood iron method of Wong. Their relative accuracy was then calculated.

(2) The average variation of these methods from the blood iron method was 3.4 per cent with the Newcomer, 2.7 per cent with the Sahli, and 3.9 per cent with the Dare.

(3) It is concluded that the Newcomer and Sahli methods are sufficiently accurate for clinical work when they are calibrated and the tests carefully performed. The Dare method is less satisfactory because of the greater possibilities for error.

REFERENCES

(1) Brown, G. E., and Roth, M.: Prognostic value of anemia in chronic glomerular nephritis. Jour. Am. Med. Assn., **81:** 1948–1950. 1923.

(2) Cullen, G. E.: A graphic representation of hemoglobin and red blood cell count ratios. Jour. Am. Med. Assn., **98:** 1067–1069. 1932.

(3) HADEN, R. L.: A clinical model of the Haden-Hausser hemoglobinometer. Jour. Lab. and Clin. Med., **18:** 1062–1065. 1933.

(4) HADEN, R. L.: The determination of the hemoglobin by the iron content method. Jour. Lab. and Clin. Med., **19:** 406–410. 1934.

(5) HASKINS, H. D.: A new permanent standard for Sahli's hemoglobinometer. Jour. Biol. Chem., **57:** 111–113. 1923.

(6) HOUGHWOUT, F. G.: A source of inconstant error in the Dare hemoglobinometer. Jour. Lab. and Clin. Med., **14:** 659–663. 1928–29.

(7) KARSHAN, M., AND FREEMAN, R. G., Jr.: A study of hemoglobin methods. Jour. Lab. and Clin. Med., **15:** 74–78. 1929.

(8) OSGOOD, E. E., AND HASKINS, H. D.: A new permanent standard for estimation of hemoglobin by the acid hematin method. Jour. Biol. Chem., **57:** 107–110. 1923.

(9) SANFORD, A. H., AND SHEARD, CHARLES: The determination of hemoglobin with the photoelectrometer. Jour. Lab. and Clin. Med., **15:** 483–489. 1930.

(10) WINTROBE, M. M.: Blood of normal young women residing in a subtropical climate—red cells, hemoglobin, volume of packed red cells, color index, volume index and saturation index. Arch. Int. Med., **45:** 287–301. 1930.

(11) WINTROBE, M. M.: Blood of normal men and women, erythrocyte count, hemoglobin and volume of packed red cells of 220 individuals. Johns Hopkins Hosp. Bull., **53:** 118–130. 1933.

(12) WONG, SAN YEN: Colorimetric determination of iron and hemoglobin in blood, II. Jour. Biol. Chem., **77:** 409–412. 1928.

THE PRESENCE OF ARSENIC IN THE BRAIN AND ITS RELATION TO PERICAPILLARY HEMORRHAGES OR SO-CALLED ACUTE HEMORRHAGIC ENCEPHALITIS*

ARNOLD E. OSTERBERG AND JAMES W. KERNOHAN

Respectively of Section on Biochemistry and Section on Pathologic Anatomy, The Mayo Clinic, Rochester, Minnesota

The presence of arsenic in any organ of the body in amounts greater than a mere trace can be considered abnormal. However, with increased usage, in the treatment of disease, of preparations containing arsenic, its presence in various tissues is to be expected in these cases. Arsenic in organic combination, and in various forms, exerts, in a few cases, a deleterious action on the hemapoietic system and, in still a smaller number of cases, a specific action on the capillaries of the white matter of the brain and of the spinal cord that leads to hemorrhage.

The condition in which miliary hemorrhages occur in the white matter of the brain and of the spinal cord following administration of some organic compounds containing arsenic frequently has been referred to as acute hemorrhagic encephalitis. The hemorrhages are limited almost exclusively to the white matter, the gray matter being practically unaffected. This finding is not usual in any recognized form of encephalitis. On histologic examination there is no evidence of inflammatory reaction. This was recognized by Globus and Ginsberg,[1] in 1933, when they suggested the term "pericapillary encephalorrhagica (due to arsphenamine)"; they thus excluded the inflammatory element and recognized the true cause of the lesion.

We are presenting four cases in which patients died following administration of organic compounds containing arsenic, as the

* Read before the Thirteenth Annual Convention of the American Society of Clinical Pathologists, Cleveland, Ohio, June 8 to 11, 1934.

result of pericapillary hemorrhage of the white matter of the brain (cases 1 to 4 inclusive, table 1) and a series of cases also in which organic compounds containing arsenic had been administered for the treatment of syphilis of the central nervous system but in which patients died from various other complications (cases 5 to 12, inclusive). These two groups of cases are compared with a group in which the subjects had ingested various inorganic arsenical compounds (cases 13 to 18, inclusive) and also with a group of five control cases in which there was no known history of administration of arsenic in any form (cases 19 to 23, inclusive).

The various types of complication resulting from administration of arsenical compounds may follow only two small doses, but more frequently they occur following several administrations of the drug. The complications listed in table 1 are seen following administration of all common types of organically bound arsenic used in the treatment of neurosyphilis. The time between onset of the complication, and death of the patient, varied from one to twenty-three days, and in cases of pericapillary hemorrhages of the white matter this time was decreased to from one to five days.

The age and sex of the patient was of no apparent significance. The determination of the arsenic content of the brain and liver was carried out by the electrolytic method of Gutzeit which was previously described by one of us (Osterberg[2]). Arsenic was more abundant in the white matter than in the gray matter of the brain in the four cases in which there were pericapillary hemorrhages (cases 1 to 4, inclusive). In these cases, the arsenic content of the liver varied greatly. In the group with other complications, the liver contained much larger amounts of arsenic than the brain. In cases 3 and 4, the patients suffered from and had been treated elsewhere for neurosyphilis, and the patient in case 2 had taken sulpharsphenamine of her own accord; consequently, it was impossible to determine the amount of arsenic these three patients had had.

At necropsy, the appearance of the brain in cases 1 to 4, inclusive, was most remarkable. The white matter of the entire cerebrum, midbrain, pons, cerebellum, and even of the pyramidal

TABLE 1

CASE	AGE	SEX	DIAGNOSIS	TYPE OF ARSENIC	CAUSE OF DEATH	ARSENIC, MGM. PER 100 GRAMS FIXED TISSUE	
						In liver	In brain
	years						
1	32	F.	Secondary syphilis	Neoarsphenamine 0.3 gm.; arsphenamine 0.3 gm.	Pericapillary hemorrhages of brain	0.125	0.120
2	36	F.	Syphilophobia	Sulpharsphenamine	Pericapillary hemorrhages of brain		0.200
3		M.	Intermittent hypertension	Unknown	Pericapillary hemorrhages of brain	Negative	0.180
4			Malignant hypertension	Unknown	Pericapillary hemorrhages of brain	0.135	0.125
5	58	M.	Tabes dorsalis	Sulpharsphenamine; tryparsamide; neoarsphenamine	Purpura hemorrhagica	0.53	0.300
6	48	M.	Neurosyphilis	Unknown (eight treatments)	Thrombosis basilar artery and infarction of pons	0.360	0.094
7	49	M.	Neurosyphilis	Arsenobenzol	Purpura hemorrhagica; aplastic anemia	0.375	0.080
8	53	F.	Latent syphilis	Sulpharsphenamine 2.3 gm. in 1923, 0.1 gm. in 1926	Acute hemorrhagic purpura	0.045	Negative
9	20	F.	Hereditary syphilis	Arsphenamine; neoarsphenamine	Purpura hemorrhagica; aplastic anemia	0.110	Negative
10	45	M.	Neurosyphilis, paresis	Tryparsamide	Hyperpyrexia	0.350	0.060
11	52	F.	Neurosyphilis	Arsphenamine; tryparsamide	Subacute yellow atrophy of liver	Negative	0.060
12	38	M.	Neurosyphilis, tabes	Arsenobenzol	Cirrhosis of liver	0.450	0.404
13	63	M.	None made	Inorganic (stomach washings)	Chronic arsenical poisoning	0.061	0.350

14	50	F.	(Suicide)	Inorganic (found in stomach)	Acute gastritis (arsenic)	1.02	0.125
15	11*	F.		Inorganic	Acute arsenical poisoning and sepsis	1.7 0.250	Trace
16	4	M.	Acute poliomyelitis	Inorganic (accidental)	Acute gastritis (arsenical poisoning)	0.138	0.375
17	4	M.		Inorganic (accidental)	Arsenical poisoning	0.044	0.080
18			(Suicide)	Inorganic	Arsenic	1.00	0.25
19	11*	F.	Acute encephalomyelitis	Unknown	Acute encephalomyelitis (hemorrhagic)	0.740	Negative
20	76	F.	Traumatic contusion of brain	Unknown	Multiple hemorrhages of brain		Negative
21			Erysipelas	Unknown	Erysipelas and sepsis	Negative	Negative
22			Septic pharyngitis	Unknown	Septic pharyngitis	0.36	Negative
23			Bronchopneumonia	Unknown	Bronchopneumonia	0.45	Negative

* Months.

tracts of the medulla oblongata was infiltrated with innumerable small hemorrhages (fig. 1). Occasionally these small hemorrhages appeared to have fused and a large hemorrhage had occurred; microscopically, however, small hemorrhages were still recognizable. The gray matter did not reveal any hemorrhages, and those in the white matter were unusual. In the center of

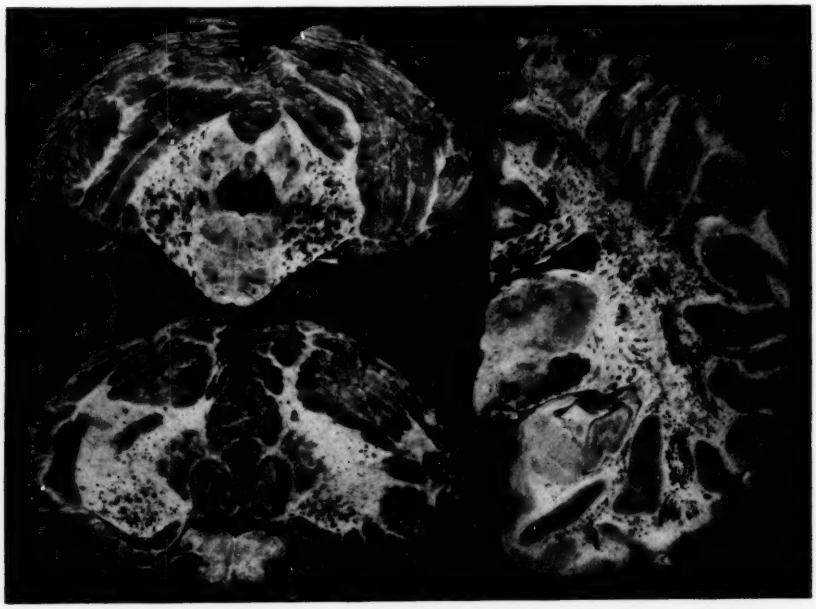

FIG. 1. PETECHIAL HEMORRHAGES IN THE WHITE MATTER OF THE CEREBRUM AND CEREBELLUM, EVEN IN THE PYRAMIDAL TRACTS OF THE MEDULLA

The gray matter of the cortex and basal nuclei have escaped the hemorrhages

each hemorrhage was a capillary filled with an eosin-staining, hyalin-like thrombus; the endothelial cells of the intima usually were prominent. Around this small blood vessel there was a zone of glial tissue free from erythrocytes, and around this zone of glial tissue was a wide ring of erythrocytes in an excellent state of preservation, without any sign of degeneration or of formation of blood pigment (fig. 2). In several regions in which

hemorrhages had fused, masses of polymorphonuclear leukocytes were present, but this seemed to us to be the result of destruction of brain tissue and not the cause of hemorrhage. In no other part of the white matter of the brain was there any evidence of acute or even of chronic inflammation.

FIG. 2. TYPICAL PETECHIAL HEMORRHAGE

Thrombosed capillary in cortex, surrounded by a zone of glial tissue. The erythrocytes form a ring outside the glial zone (hematoxylin and eosin × 160).

The arsenic content of the brain and of the liver in cases in the group in which the subjects had taken inorganic arsenic, either accidentally or with suicidal intent, were determined (cases 13 to 18, inclusive). In these cases hemorrhages in the central nervous system did not occur, yet the brain contained greater

amounts of arsenic than was found in the brain in cases in which organic arsenic had been administered or in which the patients had died from cerebral hemorrhage or from some other complication. The liver in these cases also contained varying, and usually large, amounts of arsenic.

The body of a child who had died of true hemorrhagic encephalitis was examined; the brain contained no arsenic, but the liver contained a large amount, the source of which could not be traced. Microscopic study of the brain in this case (case 19) revealed an acute inflammatory condition of the brain both in the gray and in the white matter. In the other four of these control cases (cases 20 to 23, inclusive) no arsenic was found in the central nervous system, but the amount in the liver varied considerably. The presence of arsenic in the liver cannot always be explained, but obviously there are numerous sources from which it might have been obtained.

SUMMARY

The condition known as acute hemorrhagic encephalitis frequently is not the result of an inflammatory condition, but may be due to the administration of organic compounds containing arsenic. The remarkable appearance of the brain and spinal cord is due to multiple capillary hemorrhages in the white matter of the central nervous system. Chemical determination gives evidence that arsenic is present in this tissue in relatively large amounts. When organically bound arsenic is administered in the treatment of neurosyphilis, it usually is demonstrable in the central nervous system in varying amounts. Inorganic arsenic, when ingested by accident or with suicidal intent, also accumulates in the central nervous system, but it seldom produces hemorrhages. We suggest that, in the presence of an unexplained gross hemorrhage or of multiple petechial hemorrhages in the white matter of the central nervous system, a chemical investigation for arsenic be carried out, since the presence of relatively large amounts of this element may explain the cause of the hemorrhages.

REFERENCES

(1) GLOBUS, I. H. AND GINSBERG, S. W.: Pericapillary encephalorrhagica due to arsphenamine (so-called arsphenamine encephalitis). Arch. Neurol. and Psychiat. **30:** 1226–1247. 1933.

(2) OSTERBERG, A. E.: A modification of the electrolytic Gutzeit apparatus for the estimation of arsenic in biological material. Jour. Biol. Chem. **76:** 19–22. 1928.

BLOOD DYSCRASIAS

A Symptom Complex Rather Than a Disease Entity*

B. MARKOWITZ

Sloan Clinic, Bloomington, Illinois

Discussion has arisen within recent years with regard to the etiology and classification of the various so called blood dyscrasias. Ewing's[1] discussion indicated that infection and neoplasm seem to be intricately associated with the leukemias. Keim[4] suggested that various forms of lymphadenosis listed under the diseases of lymphatic origin should be termed lymphoblastomas. In a former paper I[5] expressed the opinion that leukemias and other blood dyscrasias showed evidence of being inflammatory rather than neoplastic in origin. An unusual disease of the bone marrow was recently reported[2] in which there were marked bone marrow changes, a symptomatic granulocytopenia, and a persistent increased temperature. Careful and continued observation, and a post mortem examination failed to reveal any evidence suggestive of any known clinical entity. Jaffe[3] feels that each of these diseases is only a symptom complex produced by an outside stimulation or a faulty body reaction to such a stimulus.

Similar disease producing factors seem to elicit various responses. Blood pictures seemingly related to leukemia are often found in severe infections. The occasional blood findings of dye poisoning also simulate these leukemic phases of severe infection while the blood picture of granulocytopenia is frequently found following the use of any of the benzene ring derivatives. Both lymphatic leukemia and granulocytopenia not infrequently follow liver destruction caused by salvarsan. Granulocytopenia and aplastic anemia, too, seem to be definitely related and differ pathologically only in the extent of involvement found in the formative tissues.

* Read by title before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

In the following case reports this intricate relationship of cellular pathology with the various apparent stimuli is illustrated.

CASE REPORTS

Case 1. Mrs. R., aged 43 years, with a history of phlebitis for two years, complained of pain in the upper left abdominal region. Examination revealed a huge spleen but blood findings did not show any evidence of leukemia. Because of the great discomfort, the spleen which weighed 1,475 grams (normal, 150 grams), was removed. Microscopic examination revealed definite evidence of leukemia. Two months later the leukocytes numbered 18,000 with 58 per cent lymphocytes and an occasional immature cell.

The case was diagnosed as aleukemic leukemia associated with an inflammatory process and an occasional immature cell found only after splenectomy.

Case 2. Mr. J. M., aged 70 years, complained of rheumatism beginning two years earlier. His leukocytes numbered 13,000 with no immature cells and his spleen was somewhat enlarged. He had been to the Mayo Clinic where his leukocytes ranged from 18,000 to 30,000 with no immature cells but a relative lymphocytosis of 45 to 63 per cent. At that time a possible lymphatic leukemia was considered. His condition became progressively worse, and just before death his leukocytes numbered 16,800 with 70 per cent lymphocytes and no immature cells. Post mortem examination revealed very marked leukemic infiltrations in all the organs.

A diagnosis of aleukemic leukemia associated with some inflammatory process showing no immature cells even up to time of death, was made.

Case 3. R. R. B., a boy aged 7 years, was suddenly taken ill, vomited, and was markedly cyanosed. His leukocytes numbered 14,000 with 68 per cent lymphocytes and an occasional immature cell. Because of the definite history a diagnosis of shoe dye poisoning was made; he recovered within a few days. His blood count gradually returned to normal.

This was a case of dye poisoning with a blood count simulating case 1 a definitely proved leukemia.

Case 4. Miss R., aged 23 years, became jaundiced after intravenous injection of neosalvarsan. The blood count revealed 32,000 leukocytes with 70 per cent lymphocytes, many of which were immature. Repeated leukocyte counts ranged between 30,000 and 50,000 with increasing numbers of immature lymphocytes. The patient failed to return but the blood findings justified the diagnosis of lymphatic leukemia following neosalvarsan injection.

A leukemic blood picture following administration of neosalvarsan, was diagnosed.

Case 5. E. M., a man aged 19 years, was given anti-luetic treatment, and after the second intravenous injection of salvarsan ran the usual course of a severe reaction. His leukocytes numbered 7,000 with 52 per cent neutrophiles. Within a week he became jaundiced and his leukocytes kept dropping, ranging between 2,000 and 3,000 with granulocytes numbering 8 to 20 per cent. He was critically ill, but rallied and finally recovered completely. It may be questioned whether or not he had a true granulocytopenia but it is certain that there was a neutropenic phenomenom operating as part of the reaction to the neosalvarsan administration.

A neutropenic blood picture following administration of neosalvarsan was diagnosed.

Case 6. C. B., a man aged 27 years complained of a severe gangreneous sore throat. His leukocyte count numbered 3,000, only 20 per cent of which were leucocytes; a diagnosis of granulocytopenia was made. During the second week of illness the leukocytes rose to 7,000 with many immature leucocytes. The diagnosis was changed to leukemia. The day before death, which occurred at the end of the sixth week of illness, the total leukocytes were 22,000 with only 18 per cent mature and 50 per cent immature leukocytes. Post mortem examination of the spleen (weight, 2200 grams) and bone marrow revealed definite evidence of leukemia.

This proved to be a case of leukemia which began with typical symptoms of granulocytopenia.

CONCLUSION

The individual reactions in the form of various manifestations of so called blood dyscrasias which seem to be prompted by various stimuli in these cases indicate that each of these diseases is not in itself a disease entity but rather a symptom complex due to an outside stimulus or to some abnormal body constituent or a combination of both.

REFERENCES

(1) Ewing, J.: Neoplastic diseases. Philadelphia: W. B. Saunders, p. 375, 1919.

(2) Grulee, C. G., Appelbach, C. W., and LeMaster, R. E.: An unusual disease of the bone marrow (Osseomyelodysplasia). Jour. Am. Med. Assn., **100:** 162–167. 1933.

(3) Jaffé, R. H.: Personal communication.

(4) Keim, H. L.: Universal leukemia cutis. Arch. Derm. and Syph., **10:** 579–598. 1924.

(5) Markowitz, B.: The hematopoietic system and infection. Am. Jour. Clin. Path., **2:** 449–455. 1932.

EDITORIAL

Artificial Fever

It is apparently a human instinct to resist change, even though it is one of the certainties of life. Some misguided economists, whose theories were washed away by the economic tidal wave which inundated us some five years ago, have pleaded for a moratorium on all scientific research for a decade. Their plea is based upon the assumption that progress would cease during that time.

For centuries physicians regarded fever as an alarming symptom. Pathologists, following Virchow's teachings, ascribed various degenerative tissue changes to the effect of fever. Antipyretic drugs were almost universally employed to combat fever. From time to time, courageous observers ventured the unorthodox belief that fever was Nature's mechanism of defense against infection. In 1917 Wagner-Jauregg completely upset the teachings of centuries by demonstrating that artificially-induced fever was capable of overcoming the ordinarily disastrous effects of syphilis of the central nervous system. In the short period which has lapsed since this discovery, evidence has accumulated which makes it quite apparent that fever exerts an adverse influence upon the growth of bacteria, diminishes the potency of toxins, favors phagocytosis, and stimulates the development of immune bodies.

Wagner-Jauregg's success in malaria therapy of dementia paralytica was soon repeated by investigators in all parts of the world. The inherent dangers of engrafting one serious disease upon another as a therapeutic measure naturally led to a diligent search for a less hazardous method of producing the same effect. Comparable results were obtained by other workers following inoculations with the organisms of rat-bite fever and relapsing fever. Repeated injections of typhoid vaccine and other foreign

proteins appeared to be successful in those cases in which sustained high fever was induced. It became more and more apparent that simple fever production was the one factor common to all of these methods. These observations stimulated a demand for physical methods for artificial fever production.

The prolonged hot bath has been employed in the treatment of various infectious diseases since the time of the Greek priest-physicians. Laymen have clung tenaciously to their confidence in the curative merits of external heat. Schamberg and Tseng (1927), and Mehrtens and Pouppirt (1929) reintroduced the prolonged hot bath for thermotherapy. Rosanoff (1928) revived the outmoded hot air method. Neymann and Osborne, King and Cocke, and Whitney introduced the use of high frequency electrical currents (diathermy and radiothermy). In the past four years many other types of apparatus have been devised.

While thus far most of the emphasis has been placed on the development of apparatus, those engaged in this field have become aware of great gaps in our knowledge of the indications for and the limitations of artificial fever therapy. History is repeating itself in the attempts of certain manufacturers to exploit the field by utilizing modern high-pressure sales methods. Some of the apparatus now available is inadequate and dangerous, and is sold to any physician without thought of adequate training of the supervising physician and his technical assistants. In the hands of physicians who have familiarized themselves with the medical and physical principles involved, with the assistance of intelligent nurse-technicians who have received special training, some of the machines now available are capable of producing sustained high fever (104° to 106.8°F. for five or more hours) with safety. The undertaking is in many respects comparable to a major surgical operation, particularly as regards the necessity for a careful diagnostic survey to determine eligibility and the constant attention to the patient during the long treatment. Neglect of contra-indications will lead to disaster. It is well to emphasize the obvious, but too often disregarded, fact that skill of personnel far transcends in value the perfection of matériel. Until further developed, artificial fever therapy should be re-

stricted to institutions. Otherwise an important adventure in therapeutics is almost certainly doomed to a period of discredit, similar to that which followed the introduction of roentgen-rays.

Fundamental research is urgently needed in this field. Little is known of the physiology of fever. Fever has never been adequately defined. The splendid preliminary studies which have been made by the small band of pioneers in this virgin field indicate that much is yet to be learned of the influence of fever on bacteria, tissues and body fluids. The thermal death-time studies made by Carpenter and his associates on *Neisseria gonorrheae* and *Treponema pallidum* indicate the great possibilities for the controlled application of fever therapy to the diseases caused by these organisms. Of equal importance, also, is the fact that these studies show that much of the available information regarding the thermal death-time of various bacterial species is erroneous. It is probable that many other pathogenic organisms will be destroyed or inhibited by sustained high temperatures.

In the fields of bacteriology, serology, immunology, hematology, biochemistry, and tissue pathology, as well as in the field of clinical medicine, the opportunities and the necessity for research are apparent. The clinical pathologist is a logical person to conduct such investigations. The present situation demands a shift of emphasis from machines to men.

—Walter M. Simpson.

NEWS AND NOTICES

Annual Convention of the American Society of Clinical Pathologists

The Thirteenth Annual Convention of the American Society of Clinical Pathologists was a most successful occasion. It was held at Cleveland, Ohio, on June 8 to 11. A two-day program of scientific papers and exhibits contained a large number of excellent items. The round table discussion on Friday evening took the form of a Clinico-pathologic conference under the direction of Dr. P. F. Morse and was enthusiastically discussed. Dr. A. C. Christie read a paper on "Some Economic Problems Common to all Branches of the Medical Profession," a report of which will be published later.

The annual banquet was held on Saturday at which time the presidential address was read by Dr. A. G. Foord and Dr. M. T. MacEachern also spoke. The address of the evening was made by Dr. H. T. Karsner on "Medieval Guilds of Medical Interest." The address was followed by a program of entertainment.

Sunday and Monday were devoted to trips around the city and environs and included a visit to and luncheon at the Cleveland Clinic.

The business meeting will be reported in another issue of the Journal except for the following items:

The election of officers results in the following:

President-Elect: F. M. Johns.

Vice President: B. S. Kline.

Executive Committee (3 years): A. G. Foord, Kano Ideda.

Board of Censors: 3 years: Stanley Reimann, H. A. Heise. 1 year: A. H. Braden.

Board of Registry (3 years): W. E. King, Asher Yaguda.

A thorough revision of the constitution and by-laws was passed.

Dr. R. R. Kracke received the Ward Burdick award.

Drs. Ludvig Hektoen and Otto Naegeli were elected honorary members and the following members were elected:

Horace Broekman Anderson, Johnstown, Pa.
W. V. Bergstrom, Binghamton, N. Y.
John L. Beven, Baton Rouge, La.
Paul Jean Breslich, Minot, N. D.
Lewis Woodbridge Brown, Newark, N. J.
Frances Pullen Elliott, San Diego, Calif.
E. B. Erskine, Parris Island, S. C.
Edward Fendrick, Irvington, N. J.
Roswell Schiedt Fidler, Columbus, Ohio
Wm. Freeman, Worcester, Mass.
Carl Goehring, Steubenville, Ohio
Samuel Alexander Goldberg, Newark, N. J.
H. Goldblatt, Cleveland, Ohio
Ernest Byron Hanan, Buffalo, N. Y.
Lewis Rowland Hill, LaGrange, Ill.
Robt. M. Holbach, Toms River, N. J.
Wilbur F. Keller, Oklahoma City, Okla.
John L. Kestel, Waterloo, Iowa
F. W. Light, Clarksburg, W. Va.
Dr. Wm. R. Mathews, Shreveport, La.
Perry J. Melnick, Chicago, Ill.
David Raymond Meranze, Philadelphia, Pa.
John Davis Paul, Philadelphia, Pa.
Raymond Fridolph Peterson, Butte, Montana
Clarence Carl Pflaum, Columbia, Missouri
Robert Burton Poling, Youngstown, Ohio
O. B. Pratt, Los Angeles, Calif.
Ernest August Pribram, Chicago, Illinois
Otto Saphir, Chicago, Ill.
Edward Lowell Saylor, Akron, Ohio
Joseph I. Schleifstein, Albany, New York
W. H. Seemann, New Orleans, La.
Frederick Wm. Shaw, Richmond, Va.
I. J. Silverman, New York, N. Y.
Louis Alexander Soloff, Philadelphia, Pa.
Abraham Trumper, Montgomery, Ala.
Herman Henry Van Horn, Harrisburg, Pa.
Stuart L. Vaughan, Buffalo, N. Y.
Emmerich Von Haam, New Orleans, La.
T. T. Walker, Watertown, N. Y.
Margaret Warwick, Buffalo, N. Y.
J. S. Weingart, Des Moines, Ia.
John Wenner, Allentown, Pa.
Corren Pinckney Youmans, St. Petersburg, Fla.

Attention of Clinical Pathologists is called to a recent decision by the supreme court re Granger v. Adson et al. (Minn. 250 N. W. 722).

Granger, a layman, conducted a so-called health audit service. For a fee, he undertook to examine urine and make blood pressure tests and to report the results. He employed a Dr. Grave, a licensed physician, to make the analyses and to report to him. Granger in turn passed on the report to the subscribers. The court held that Granger was as much practicing medicine in employing Dr. Grave to do the work for him as he would have

been if he himself had attempted to make the urine analyses and he did in making the blood pressure tests. "To pass on to the subscriber advice as to whether or not the tests indicated a normal or abnormal condition, and whether or not the subscriber should consult his physician or be content with the advice which Granger himself might give in regard to diet, exercise, and mode of living, was practicing medicine." "The law intends that the patient shall be the patient of a licensed physician, not the patient of a corporation or layman. The obligations and duties of a physician demand no less. There is no place for a middleman."

It is interesting to note that the court could not see any objection to the employment of technicians and other experts by physicians leaving the results of the work of the technician or expert to be interpreted by a physician as a help to diagnosis. The court held that Granger was practicing medicine in violation of the law.

This decision is of great importance to Clinical Pathologists for it should establish rather clearly the status of a technician, a bacteriologist, or chemist without a medical degree and not licensed to practice medicine. It clearly indicates that where such technicians or experts perform tests they must do so under the employment of physicians and that a physician must take the ultimate responsibility for the diagnosis, the report, and its interpretation.

BOOK REVIEWS

Urinary Analysis and Diagnosis by Microscopical and Chemical Examination. By Louis Heitzmann. 6th Ed. Pp. xxi + 366, 1934. Baltimore, William Wood & Co. $5.00.

The text has been revised although the original general plan of the book remains the same. Tests are given which can be performed by physicians who do not have any but simple apparatus and modest laboratories. After introductory material, the chemical examination of urine is treated along rather orthodox lines. The exacting student will miss a critical evaluation of the many tests given under each heading and statement of the degree of sensitivity of these tests. The major stress in the book is on microscopical method, with which the second part deals. The author contends that with the use of higher magnification, one can tell the source of cellular materials and hence arrive at a diagnosis of the part infected and the nature of the lesion. The third part gives the application of the theory to clinical entities. The numerous illustrations are free hand pen and ink sketches, most of them very crude and not accurate as to size. Those illustrating parasitic forms are without value, and the discussion of *Trichomonas vaginalis* is inexcusable; that dealing with actinomyces is misleading and inaccurate. Dr. Dannreuther has contributed a chapter on functional kidney tests and the book closes with a chapter on hormone pregnancy tests.

The book may be of value to the clinician who lacks a well organized laboratory but the clinical pathologist will find it antiquated, limited, and below the standard of many modern texts.

Brucella Infections in Animals and Man. By I. Forest Huddleson. Pp. xvi + 108. New York, The Commonwealth Fund.

This is an important summary of methods of laboratory diagnosis in brucellosis. There is first an historical discussion of the genus Brucella which includes the general characteristics of the

three species. This is followed by a chapter dealing with methods of isolating the organism from cattle, from the milk of animals, from man, and from the tissues of animals, with details as to methods in which inoculation of guinea pigs is employed. The pathology of the infection in experimental guinea pigs and in natural infections of man and animals is discussed. The chapter following treats of the serological methods of determining infection with particular reference to agglutination and the shortcomings of these as diagnostic methods as applied to the disease in man. Details are given for preparation and interpretation of the nucleoprotein skin test and other such tests. The author then gives in detail his opsono-cytophagic test of the blood and its interpretation combined with the nucleoprotein skin test. The final chapter is concerned with methods of differentiating the species of the genus. A reference list of 188 items concludes the text. This is a useful and comprehensive manual for laboratory workers, and those interested in experimental phases of this subject.